PATENT ABSTRACTS OF JAPAN

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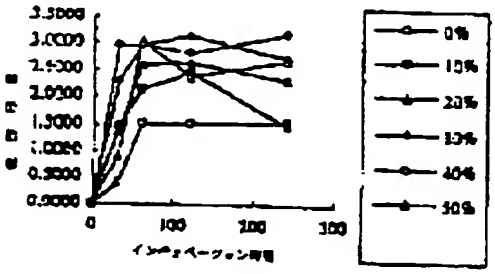
(72)Inventor : UEDA TAKEHIKO
SUNAMOTO JUNZO

(54) LIPOSOME RECONSTRUCTION TYPE INSULIN RECEPTOR

(57)Abstract:
PURPOSE: To obtain the subject new insulin receptor, useful for elucidating an information transmission system of the living body or research, etc., on treatment, diagnosis, etc., of diabetes and highly separated and purified without denaturation by directly transferring a membranous protein onto a liposome and purifying the resultant membranous protein.
CONSTITUTION: This liposome reconstruction type insulin receptor is obtained by incubating a liposome containing a cell membranous fraction purified from a bovine placental tissue and an artificial boundary lipid [dipalmitoylphosphatidylcholine (DPPC)] in a buffer solution, directly transfer the membranous protein onto the liposome and then separate a liposome fraction according to a density-gradient ultracentrifugation method. Thereby, the membranous protein is directly transferred onto the liposome and highly purified and the receptor is extremely important to the elucidation of an information transmission system in the living body and treatment, diagnosis, etc., of diseases incidental to civilization such as diabetes.



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CLAIMS

[Claim(s)]
[Claim 1] The liposome reconstruction type insulin receptor by which transferred to the membrane protein directly and it was refined on the liposome.
[Claim 2] The insulin receptor of the claim 1 which a liposome becomes from a mixed liposome.
[Claim 3] The insulin receptor of the claim 2 whose mixed liposome is DDPC.
[Claim 4] The insulin receptor of the claims 1, 2, or 3 whose membrane proteins are the cell membrane fractions from a placenta organization.
[Claim 5] The purification method of the insulin receptor which is made to transfer a membrane protein direc: and refines it to up to a liposome.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a liposome reconstruction type insulin receptor. This invention relates to the insulin receptor which enabled advanced refining by the liposome, and its purification method still in detail.
[0002]
[Description of the Prior Art] Conventionally, in connection with development of a life science, bionics, etc., advanced refining of protein has been a big technical probrem, and it has become about the technical probrem important for a body tissue, a research of a cell, and expansion of the new medicine based on those knowledge, and the iatrotechnique. However, protein had become the failure for specialization of the acquired protein, and a study of the operation and an application, in order to be accompanied by the conversion in the process of the separation and refining in **********. And for example, there was an insulin receptor as one of such protein. Although this insulin receptor was protein very important because of an elucidation of the communication-of-information system in ** and a living body, the treatment of the civilization disease of diabetes, a diagnosis, etc., it was very difficult a separation and to refine, without being accompanied by the conversion with old technique.
[0003] This invention is made in view of the situation as above, conquers the limitation of old technique, and aims at offering the new means and the insulin receptor acquired by this which attracts attention as advanced refining of protein.
[0004]
[Means for Solving the Problem] This invention offers the liposome reconstruction type insulin receptor by which transferred to the membrane protein directly and it was refined on the liposome as what solves the above-mentioned technical probrem. And this invention also offers again the purification method of the insulin receptor which is made to transfer a membrane protein directly and refines it to up to a liposome.
[0005]
[Function] Although it becomes the insulin receptor which this invention was directly transferred to the membrane protein as above-mentioned on the liposome, and was refined from the purification method, this is based on having established this as a means of advanced refining based on knowledge that protein transfers on a liposome, if a viable cell and a liposome are mixed under a culture condition.
[0006] Since direct transition of a up to [the liposome of the protein in this invention] does not need the so-called solubilization operation at all, it is not accompanied by proteinic denaturation. For this reason, it is observed as an advanced refining means. And the insulin receptor with it difficult [to acquire until now] by which advanced refining was carried out, that is, liposome reconstruction was carried out in this invention will be obtained by this refining means.
[0007] The liposome in this case is idea taken into consideration as the so-called phospholipid, and can be considered more widely. Especially, by the ability using a mixed-type liposome suitably in this invention, the mixed liposome using the artificial boundary lipid (DDPC) has high transition luminous efficacy, and gives an insulin receptor with high yield and selectivity. What is necessary is to illustrate the cell membrane fraction from a placenta organization, for example, and just to cultivate the liposome containing this cell membrane fraction, DDPC, etc. in the buffer solution in case of refining by the liposome as a target membrane protein. Mixed proportion with a liposome can be made into about 1 / ten to 4/5, for example as the weight ratio, and the buffer solution can usually be used for incubation in about ordinary temperature -70 degree C] temperature.
[0008] About the buffer solution, it cannot be overemphasized that the suitable composition including a well-known thing is employable. Moreover, about a candidate organization, various kinds of things, such as a cow and the swine, are taken into consideration. Hereafter, an example is shown and this invention is explained still in detail.
[0009]
[Example] The cell membrane fraction refined from the cow placenta organization and the liposome containing DDPC were incubated in the buffer solution. Then, the liposome fraction was separated by the density gradient ultracentrifugation. It was checked by the Western-blotting method using the insulin which carried out the indicator that the insulin receptor subunit has transferred to this fraction.
[0010] It turns out that it depends for it to DDPC content of a liposome strongly as the transition luminous efficacy of a membrane protein was illustrated to drawing 1 . Moreover, the transition speed was also understood that the parvus thing of the molecular weight of a membrane protein is in the inclination to be quick.
[0011]
[Effect of the Invention] A liposome and a reconfigurated type insulin receptor are offered by this invention as advanced refining accompanied by proteinic denaturation as explained in detail above. An application of the knowledge will be expected by this receptor with an elucidation of a cell communication-of-information system etc.

[Translation done.]

* NOTICES *

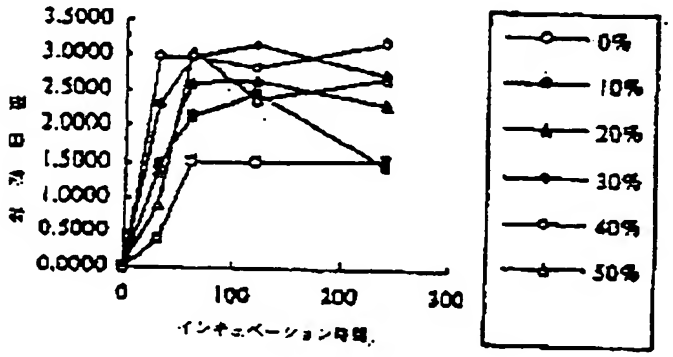
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing which illustrated the protein transition luminous efficacy as an example.

[Translation done.]

DRAWINGS

[Drawing 1]



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* NOTICES *

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[Procedure revision]
[Filing Date] April 6, Heisei 7
[Procedure correction 1]
[Document to be Amended] Specification
[Item(s) to be Amended] Whole sentence
[Method of Amendment] Change
[Proposed Amendment]
[Document Name] Specification
[Title of the Invention] Liposome reconstruction type insulin receptor
[Claim(s)]
[Claim 1] The liposome reconstruction type insulin receptor by which transferred to the membrane protein directly and it was refined on the liposome.
[Claim 2] The insulin receptor of the claim 1 which a liposome becomes from a mixed lipid liposome.
[Claim 3] The insulin receptor of the claim 2 whose composition of a mixed lipid liposome is DDPC and DMPC.
[Claim 4] The insulin receptor of the claims 1, 2, or 3 whose membrane proteins are the things of the cell membrane fraction origin from a placenta organization.
[Claim 5] The purification method of the insulin receptor which is made to transfer a membrane protein directly and refines it to up to a liposome.
[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a liposome reconstruction type insulin receptor. This invention relates to the insulin receptor which enabled advanced refining by the liposome, and its purification method still in detail.
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[Description of the Prior Art] Conventionally, in connection with development of a life science, bionics, etc., advanced refining of protein has been a big technical probrem, and it has been a technical probrem important for a body tissue, a research of a cell, and expansion of the new medicine based on those knowledge, and the iatrotechnique. However, especially, the membrane protein had become the failure for specialization of the acquired protein, and a study of the operation and an application, in order to be accompanied by denaturation in the process of the separation and refining in almost all cases. And for example, there was an insulin receptor as one of such membrane proteins. Although this insulin receptor was protein very important because of an elucidation of the communication-of-information system in a living body, the treatment of the civilization disease of diabetes, a diagnosis, etc., a separation and refining were very difficult a receptor, without being accompanied by the conversion with old technique.
[0003] This invention is made in view of the situation as above, conquers the limitation of old technique, and aims at offering the new means and the insulin receptor acquired by this which attracts attention as an advanced purification method of a membrane protein.
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[Means for Solving the Problem] This invention offers the liposome reconstruction type insulin receptor by which transferred to the membrane protein directly and it was refined on the liposome as what solves the above-mentioned technical probrem. And this invention also offers again the purification method of the insulin receptor which is made to transfer a membrane protein directly and refines it to up to a liposome.
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[Function] Although it becomes the insulin receptor which this invention was directly transferred to the membrane

protein as above-mentione[redacted]he liposome, and was refined from the purificati[redacted]thod, this is based on having established this as a means [redacted]dvanced refining based on knowledge that cell membrane protein transfers on a liposome layer, if a viable cell and a liposome are mixed under a culture condition.
[0006] Since direct transition of a up to [the liposome of the membrane protein in this invention] does not need the so-called solubilization operation at all, it is not accompanied by the denaturation of a membrane protein. For this reason, it is observed as an advanced refining means. And it means that the insulin receptor with it difficult [to acquire until now] refined highly had been reconfigurated by this refining means at the liposome.
[0007] The liposome in this case is idea taken into consideration as a vesicle with the so-called phospholipid dyad layer, and can be considered more widely. The mixed lipid liposome which could use the mixed lipid liposome suitably in this invention, for example, contained the artificial boundary lipid (DDPC) especially has high transition luminous efficacy, is reconfigurated with high yield and selectivity and gives an insulin receptor. What is necessary is to illustrate the cell membrane fraction from a placenta organization, for example, and just to process the liposome containing this cell membrane fraction, DDPC, etc. in the buffer solution in case of refining by the liposome as a target membrane protein. Mixed proportion with a liposome can be made into about 1 / ten to 4/5, for example as the weight ratio, and the buffer solution can usually be used for processing in about [ordinary temperature -37 degree C] temperature.
[0008] About the buffer solution, it cannot be overemphasized that the suitable composition including a well-known thing is employable. Moreover, about a candidate organization, various kinds of things, such as the cows including the Homo sapiens and the swine, are taken into consideration. Hereafter, an example is shown and this invention is explained still in detail.
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[0010] It turns out that it depends for it to DDPC content of a liposome strongly as the transition luminous efficacy of a membrane protein was illustrated to drawing 1. Moreover, the transition speed was also understood that the parvus thing of the molecular weight of a membrane protein is in the inclination to be quick.
[0011]
[Effect of the Invention] A liposome reconstruction type insulin receptor is offered by this invention as an advanced purification method accompanied by the denaturation of cell membrane protein as explained in detail above. An application of the knowledge will be expected by this receptor with an elucidation of a cell communication-of-information system etc.
[Brief Description of the Drawings]
[Drawing 1] It is drawing which illustrated the membrane-protein transition luminous efficacy as an example.

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[a procedure revision]
[Filing Date] February 2, Heisei 10
[Procedure correction 1]
[Document to be Amended] Specification
[Item(s) to be Amended] Whole sentence
[Method of Amendment] Change
[Proposed Amendment]
[Document Name] Specification
[Title of the Invention] Liposome reconstruction type insulin receptor
[Claim(s)]
[Claim 1] The liposome reconstruction type insulin receptor in which the membrane protein was directly extracted by the liposome and was formed.
[Claim 2] The insulin receptor of the claim 1 which a liposome becomes from a mixed lipid liposome.
[Claim 3] The insulin receptor of the claim 2 whose composition of a mixed lipid liposome is DnDPC and DMPC.
[Claim 4] The insulin receptor of the claims 1, 2, or 3 whose membrane proteins are the things of the cell membrane fraction origin from a placenta organization.
[Claim 5] The purification method of the insulin receptor from which a membrane protein is directly extracted by the liposome.
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[0003] This invention is made in view of the situation as above, conquers the limitation of old technique, and aims at offering the new means which attracts attention as an advanced purification method of a membrane protein, and the insulin receptor acquired by this.
[0004]

[Means for Solving th[redacted]blem] This invention offers the liposome reconst[redacted]n type insulin receptor which the membrane protein was [redacted]tracted from the direct cell membrane by presenc[redacted] liposome, and was formed as what solves the above-mentioned technical probrem. And this invention also offers again the purification method of the insulin receptor which is made to reconfigurate a membrane protein directly and refines it to up to a liposome.
[0005]
[Function] It is based on having established this as a means of advanced refining based on knowledge although it consists of a liposome reconstruction insulin receptor which the induction of the membrane protein as the above this invention] was carried out by the liposome, was extracted directly, and was formed, and its purification method, if this mixes a viable cell and a liposome under a culture condition, that the induction of the cell membrane protein will be carried out by the liposome and it will be extracted.
[0006] Since the direct extraction by the liposome of the membrane protein in this invention does not need the solubilization operation by the so-called surfactant etc. at all, it is not accompanied by the denaturation of a membrane protein. For this reason, it is observed as an advanced refining means. And the insulin receptor with it difficult [to acquire until now] refined highly will be reconfigurated by this refining means at a liposome.
[0007] The liposome in this case is idea taken into consideration as a vesicle with the so-called phospholipid dyad membrane structure, and can be considered more widely. The mixed lipid liposome which could use the mixed lipid liposome suitably in this invention, for example, contained the artificial boundary lipid (DnDPC) especially has a high extraction efficiency, it is reconfigurated by the liposome with high selectivity and an insulin receptor is given. What is necessary is to illustrate the cell membrane fraction from a placenta organization, for example, and just to process the liposome containing this cell membrane fraction, DnDPC, etc. in the buffer solution in case of refining by the liposome as a target membrane protein. Mixed proportion with a liposome can be made into about 1 / ten to 4/5, for example as the weight ratio, and the buffer solution can usually be used for processing in about [ordinary temperature -37 degree C] temperature.
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[0010] It turns out that it depends for it to DnDPC content of a liposome strongly as extraction / reconstruction luminous efficacy of a membrane protein was illustrated to drawing 1. Moreover, the extraction speed was also understood that the parvus thing of the molecular weight of a membrane protein is in the inclination to be quick.
[0011]
[Effect of the Invention] A liposome reconstruction type insulin receptor is offered by this invention as an advanced purification method accompanied by the denaturation of cell membrane protein as explained in detail above. An application of the knowledge will be expected by this receptor with an elucidation of a cell communication-of-information system etc.
[Brief Description of the Drawings]
[Drawing 1] It is drawing which illustrated the membrane-protein transition luminous efficacy as an example.

[Translation done.]

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特開平7－267996

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1/14		8318-4H		

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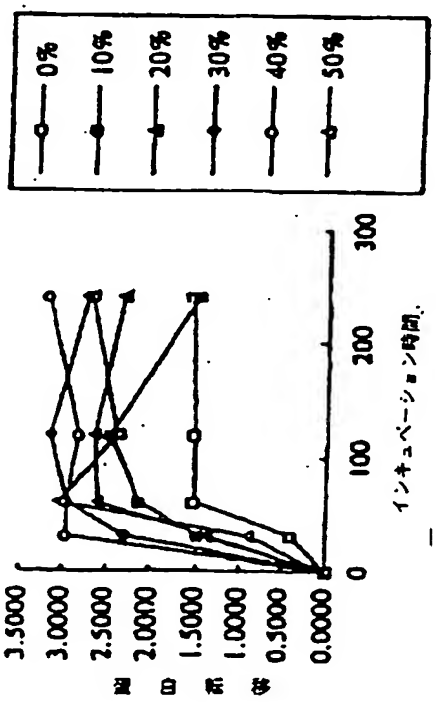
(54)【発明の名称】リポソーム再構成型インスリンレセプター

(57)【要約】
【目的】　高度に精製されたインスリンレセプターと、そのための精製方法を提供する。
【構成】　膜蛋白質がリポソーム上へ直接転移されて精製されたリポソーム再構成型のインスリンレセプター。



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【特許請求の範囲】

【請求項１】　膜蛋白質がリポソーム上へ直接転移されて精製されたリポソーム再構成型インスリンレセプター。

【請求項２】　リポソームが混合リポソームからなる請求項１のインスリンレセプター。

【請求項３】　混合リポソームがＤＤＰＣである請求項２のインスリンレセプター。

【請求項４】　膜蛋白質が胎盤組織からの細胞膜画分である請求項１、２、または３のインスリンレセプター。

【請求項５】　膜蛋白質をリポソーム上へ直接転移させて精製するインスリンレセプターの精製法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】この発明は、リポソーム再構成型インスリンレセプターに関するものである。さらに詳しくは、この発明は、リポソームによる高度精製を可能としたインスリンレセプターと、その精製法に関するものである。

【０００２】

【従来の技術とその課題】従来より、生命科学、生物工学等の発展にともなって、蛋白質の高度精製が大きな課題になっており、生体組織や細胞の研究や、それらの知見を踏まえた新しい医学、医療技術の展開にとって重要な課題をなっている。しかしながら、蛋白質はほとんどんの場合、その分離、精製の過程において変成をともなうため、取得された蛋白質の特定や、その作用、並びに応用の検討にとっての障害となっていた。そして、たとえば、このような蛋白質の一つとしてインスリンレセプターがあった。このインスリンレセプターはは、生体における情報伝達系の解明や、糖尿病という文明病の治療、診断等のために大変に重要な蛋白質であるが、これまでの技術では、その変成をともなうことなく分離・精製することは極めて困難であった。

【０００３】この発明は、以上の通りの事情に鑑みてなされたものであって、これまでの技術の限界を克服し、蛋白質の高度精製として注目される、新しい手段と、これによって取得されるインスリンレセプターを提供することを目的としている。

【０００４】

【課題を解決するための手段】この発明は、上記の課題を解決するものとして、膜蛋白質がリポソーム上へ直接転移されて精製されたリポソーム再構成型インスリンレセプターを提供する。そしてまた、この発明は、膜蛋白質をリポソーム上へ直接転移させて精製するインスリンレセプターの精製法をも提供する。

【０００５】

【作用】この発明は、上記の通りの、膜蛋白質がリポソーム上に直接転移されて精製されたインスリンレセプターと、その精製法からなるものであるが、このことは、生細胞とリポソームを培養条件下で混合すると蛋白質がリポソーム上に転移してくるとの知見を踏まえ、これを高度精製の手段として確立したことに基づいている。

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【０００６】この発明での蛋白質のリポソーム上への直接転移は、いわゆる可溶化操作を全く必要としないため、蛋白質の変性をともなわない。このため、高度な精製手段として注目されるものである。そして、この精製手段によって、これまで取得することが困難な、高度精製された、つまり、この発明においてリポソーム再構成されたインスリンレセプターが得られることになる。

【０００７】この場合のリポソームは、いわゆるリン脂質として考慮される概念であって、より広く考えることができる。なかでも、混合型リポソームは、この発明において好適に使用でき、たとえば人工境界脂質（ＤＤＰＣ）を用いた混合リポソームは、転移効率が高く、高い収率、選択性でインスリンレセプターを与える。対象とする膜蛋白質としては、たとえば胎盤組織からの細胞膜画分が例示され、リポソームによる精製に際しては、この細胞膜画分とＤＤＰＣ等を含むリポソームを緩衝液中で培養すればよい。培養には、リポソームとの混合比率を、たとえばその重量比として１／１０～４／５程度とし、通常は、常温～７０℃程度の温度において、緩衝液を用いることができる。

【０００８】緩衝液については、公知のものをはじめとして適当な組成が採用できることは言うまでもない。また、対象組織については、牛、ブタ等の各種のものが考慮される。以下、実施例を示し、さらに詳しくこの発明について説明する。

【０００９】

【実施例】牛胎盤組織から精製した細胞膜画分と、ＤＤＰＣを含むリポソームを緩衝液中でインキュベートした。その後、密度勾配超遠心法でリポソーム画分を分離した。この画分には、インスリンレセプターサブユニットが転移していることが、標識したインスリンを用いたウェスタン・ブロッテイング法によって確認された。

【００１０】膜蛋白質の転移効率は、図１に例示した通り、リポソームのＤＤＰＣ含有率に強く依存していることがわかる。また、転移速度は、膜蛋白質の分子量の小さいものほど速いという傾向にあることもわかった。

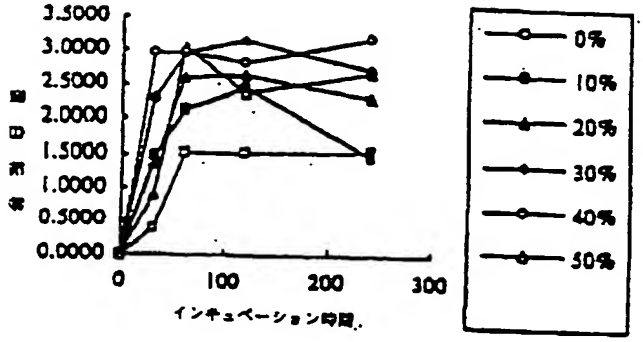
【００１１】

【発明の効果】以上詳しく説明した通り、この発明により、蛋白質の変性をともなわない高度精製として、リポソーム、再構成型のインスリンレセプターが提供される。このレセプターにより細胞情報伝達系等の解明とともに、その知見の応用が期待されることになる。

【図面の簡単な説明】

【図１】実施例としての蛋白質転移効率を例示した図である。

【図１】



【手続補正書】
【提出日】平成７年４月６日
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】全文
【補正方法】変更
【補正内容】
【書類名】　　明細書
【発明の名称】　リポソーム再構成型インスリンレセプター
【特許請求の範囲】
【請求項１】　膜蛋白質がリポソーム上へ直接転移されて精製されたリポソーム再構成型インスリンレセプター。
【請求項２】　リポソームが混合脂質リポソームからなる請求項１のインスリンレセプター。
【請求項３】　混合脂質リポソームの組成がＤＤＰＣとＤＭＰＣである請求項２のインスリンレセプター。
【請求項４】　膜蛋白質が胎盤組織からの細胞膜画分由来のものである請求項１、２、または３のインスリンレセプター。
【請求項５】　膜蛋白質をリポソーム上へ直接転移させて精製するインスリンレセプターの精製法。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】この発明は、リポソーム再構成型インスリンレセプターに関するものである。さらに詳しくは、この発明は、リポソームによる高度精製を可能としたインスリンレセプターと、その精製法に関するものである。
【０００２】
【従来の技術とその課題】従来より、生命科学、生物工学等の発展にともなって、蛋白質の高度精製が大きな課題になっており、生体組織や細胞の研究や、それらの知見を踏まえた新しい医学、医療技術の展開にとって重要な課題となっている。しかしながら、特に膜蛋白質はほとんどの場合、その分離、精製の過程において変性をともなうため、取得された蛋白質の特定や、その作用、並びに応用の検討にとっての障害となっていた。そして、たとえば、このような膜蛋白質の一つとしてインスリンレセプターがあった。このインスリンレセプターは、生体における情報伝達系の解明や、糖尿病という文明病の治療、診断等のために大変に重要な蛋白質であるが、これまでの技術では、その変成をともなうことなく分離・精製することは極めて困難であった。
【０００３】この発明は、以上の通りの事情に鑑みてなされたものであって、これまでの技術の限界を克服し、膜蛋白質の高度精製法として注目される、新しい手段と、これによって取得されるインスリンレセプターを提供することを目的としている。
【０００４】
【課題を解決するための手段】この発明は、上記の課題を解決するものとして、膜蛋白質がリポソーム上へ直接転移されて精製されたリポソーム再構成型インスリンレセプターを提供する。そしてまた、この発明は、膜蛋白質をリポソーム上へ直接転移させて精製するインスリンレセプターの精製法をも提供する。
【０００５】
【作用】この発明は、上記の通りの、膜蛋白質がリポソーム上に直接転移されて精製されたインスリンレセプターと、その精製法からなるものであるが、このことは、生細胞とリポソームを培養条件下で混合すると細胞膜蛋白質がリポソーム膜上に転移してくるとの知見を踏まえ、これを高度精製の手段として確立したことに基づいている。

【０００６】この発明での膜蛋白質のリポソーム上への直接転移は、いわゆる可溶化操作を全く必要としないため、膜蛋白質の変性をともなわない。このため、高度な精製手段として注目されるものである。そして、この精製手段によって、これまで取得することが困難な、高度に精製されたインスリンレセプターがリポソームに再構成されたことになる。

【０００７】この場合のリポソームは、いわゆるリン脂質２分子膜をもつ小胞として考慮される概念であって、より広く考えることができる。なかでも、混合脂質リポソームは、この発明において好適に使用でき、たとえば人工境界脂質（ＤＤＰＣ）を含んだ混合脂質リポソームは、転移効率が高く、高い収率、選択性で再構成されてインスリンレセプターを与える。対象とする膜蛋白質としては、たとえば胎盤組織からの細胞膜画分が例示され、リポソームによる精製に際しては、この細胞膜画分とＤＤＰＣ等を含むリポソームを緩衝液中で処理すればよい。処理には、リポソームとの混合比率を、たとえばその重量比として１／１０～４／５程度とし、通常は、常温～３７℃程度の温度において、緩衝液を用いることができる。

【０００８】緩衝液については、公知のものをはじめとして適当な組成が採用できることは言うまでもない。また、対象組織については、ヒトをはじめとして牛、ブタ等の各種のものが考慮される。以下、実施例を示し、さらに詳しくこの発明について説明する。

【０００９】

【実施例】牛胎盤組織から精製した細胞膜画分と、ＤＤＰＣを含むリポソームを緩衝液中でインキュベートした。その後、密度勾配超遠心法でリポソーム画分を分離した。この画分には、インスリンレセプターサブユニットが転移していることが、標識したインスリンを用いたウェスタン・ブロッテイング法によって確認された。

【００１０】膜蛋白質の転移効率は、図１に例示した通り、リポソームのＤＤＰＣ含有率に強く依存していることがわかる。また、転移速度は、膜蛋白質の分子量の小さいものほど速いという傾向にあることもわかった。

【００１１】

【発明の効果】以上詳しく説明した通り、この発明により、細胞膜蛋白質の変性をともなわない高度精製法として、リポソーム再構成型のインスリンレセプターが提供される。このレセプターにより細胞情報伝達系等の解明とともに、その知見の応用が期待されることになる。

【図面の簡単な説明】

【図１】実施例としての膜蛋白質転移効率を例示した図である。